

Phyto-oestrogens and risk of prostate cancer in Scottish men

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A population-based case–control study of diet, inherited susceptibility and prostate cancer was undertaken in the lowlands and central belt of Scotland to investigate the effect of phyto-oestrogen intake and serum concentrations on prostate cancer risk. A total of 433 cases and 483 controls aged 50–74 years were asked to complete a validated FFQ and provide a non-fasting blood sample. Multivariate logistic regression analysis found significant inverse associations with increased serum concentrations of enterolactone (adjusted OR 0.40, 95% CI 0.22, 0.71) and with the consumption of soy foods (adjusted OR 0.52, 95% CI 0.30, 0.91). However, no significant associations were observed for isoflavone intake or serum genistein, daidzein and equol. This study supports the hypotheses that soy foods and enterolactone metabolised from dietary lignans protect against prostate cancer in older Scottish men.

Prostate cancer: Phyto-oestrogens: Isoflavones: Lignans: Soy foods

Prostate cancer (PCa) is an important and increasing public health problem in Scotland. It is the second most common cancer in men after lung cancer, and accounts for 10% of all male cancer-related deaths (Kirk & Alexander, 2001). Within the last three decades, PCa incidence in Scotland has more than doubled (Kirk & Alexander, 2001), with 2335 cases of PCa diagnosed in 2002 (ISD Scotland, 2004). The relatively low risk of PCa in Asian populations compared with Western countries, including Scotland, suggests that dietary factors may influence the prevalence of and mortality from this disease (Armstrong & Doll, 1975; Muir *et al.* 1991; Parkin & Muir, 1992; Maskarinec *et al.* 1998). Of special interest is the group of plant-derived nutrients called phyto-oestrogens, in particular isoflavones (genistein, daidzein and equol) and lignans (enterolactone and enterodiol).

Isoflavones are found mainly in soybeans and soy products (Coward *et al.* 1993; Kuzer & Xu, 1997; Mazur, 1998; Murphy *et al.* 1999; Horn-Ross *et al.* 2000b), foods that are consumed in far greater amounts by populations in Asia compared with those in Western countries (Chen *et al.* 1999; Wakai *et al.* 1999). In Western countries, soy foods tend to be eaten most frequently by vegetarians and vegans (Chen *et al.* 1999; Arai *et al.* 2000). Isoflavone precursors are metabolised by the gut microflora to give rise to compounds such as daidzein, genistein and equol (Griffiths *et al.* 1998). Another group of phyto-oestrogens are the lignans; they are derived from the plant precursors matairesinol and secoisolariciresinol which are present in a wide variety of plant foods, including

linseed, legumes, cereals, fruits and vegetables (Mazur, 1998; Liggins *et al.* 2000c). These plant precursors are metabolised by gut microflora into the lignans enterolactone and enterodiol.

The relevant biological properties of phyto-oestrogens include antiviral, antiangiogenic and antioxidant properties (Griffiths *et al.* 1998). Phyto-oestrogens possess weak oestrogenic activity, they compete with oestradiol in binding to the nuclear oestrogen receptor and also stimulate the synthesis of sex hormone-binding globulin, which in turn mediates the plasma levels of testosterone on which the growth, development, maintenance and function of the prostate gland is dependent. In addition, phyto-oestrogens can inhibit steroid-metabolising enzymes, including 5 α -reductase and aromatase, and also the cell signalling apparatus by the inhibition of tyrosine-specific protein kinases (Griffiths *et al.* 1998).

Several epidemiological studies support the role of phyto-oestrogens and soy foods in reducing cancer risk (Adlercreutz & Mazur, 1997; Yan & Spitznagel, 2005). However, this evidence is limited, in particular for PCa, with only a few studies examining this association usually in populations with high isoflavone/soy food consumption (Mills *et al.* 1989; Severson *et al.* 1989; Jacobsen *et al.* 1998; Kolonel *et al.* 2000; Akaza *et al.* 2002; Lee *et al.* 2003; Ozasa *et al.* 2004).

The association of phyto-oestrogen intake and PCa risk has been difficult to study due to a lack of a suitable and comprehensive phyto-oestrogen food database. Recently, several phyto-oestrogen databases have been created (Pillow *et al.*

1999; USDA, 1999; Horn-Ross *et al.* 2000a; Venus Phyto-oestrogen Database, 2004). However, these databases, mainly created in the USA, are not comprehensive enough to be used to assess the dietary impact of phyto-oestrogens in the UK. This study uses a recently created and validated isoflavone food database (Ritchie, 2004; Ritchie *et al.* 2004, 2005) which is based on foods normally consumed in Scotland.

The first aim of this study was to define the distributions of the estimated dietary intake of isoflavones, and serum concentrations of the main isoflavones daidzein and genistein, the isoflavan equol and the lignan enterolactone in older men participating in the PCANDIET study, a case-control study of PCa, inherited susceptibility and diet. The second aim was to examine the association between phyto-oestrogen intake and serum concentrations and PCa. To our knowledge, this is the first time the association between phyto-oestrogens and PCa has been investigated within a UK male population.

Methods

Subjects

The PCANDIET Study, a population-based epidemiological case-control study of PCa in relation to inherited susceptibility and diet, was carried out in the lowlands and central belt of Scotland. This report focuses on the dietary side of this study, in particular phyto-oestrogens. The study population included men aged 50–74 years who resided in the Borders, Lothian and Greater Glasgow Health Board areas. Between April 1998 and December 2001, we attempted to identify all men newly diagnosed with histologically confirmed clinically important PCa (defined as grade >T1a and Gleason score >4) at hospitals within the Lothian, Borders and Glasgow Health Boards (Western General Hospital, Edinburgh; Borders General Hospital, Melrose; Glasgow Royal Infirmary; Stobhill Hospital, Glasgow; Victoria Infirmary, Glasgow; Southern General Hospital, Glasgow; Gartnavel General Hospital, Glasgow; and St John's Hospital, Livingston). Population controls, frequency matched by age and Health Board to cases, were randomly selected from Health Board Lists (Lothian and Borders Health Boards) and GP lists (Glasgow Health Board). In addition, all men newly diagnosed with benign prostatic hyperplasia (BPH) between April 1998 and December 2001 at the Western General Hospital, Edinburgh and Borders General Hospital, Melrose were identified, in order to be used as a second control group. The study was restricted to men who were mentally capable of completing a questionnaire and who had no previous diagnosis of PCa.

Subjects were invited to participate in the study after receiving permission to contact them from their consultant (cases and BPH controls) or GP (population controls). After providing written consent to participate in the study, the subjects were asked to complete the Scottish Collaborative Group FFQ (SCG-FFQ) and to provide a non-fasting blood sample.

Dietary assessment

A copy of the SCG-FFQ was posted to each subject for self-completion. Guidelines for completing the questionnaire and photographic information on the size of portions were

also included, and advice on completing the SCG-FFQ was available by FreePhone.

The SCG-FFQ has been used extensively in Scotland (Bolton-Smith *et al.* 1995; Sharp *et al.* 2002) and has been validated against serum isoflavones (Heald *et al.* 2005) and 4 d weighed diet records for adequacy of macro and some micronutrient intakes (Masson *et al.* 2003). It focused on dietary habit during the past 2–3 months and specified 155 food items accounting for 600 individual foods. For each of these food items, the respondent was asked to select from nine categories (ranging from 'rarely/never' to '7 days per week') the number of days per week the food item was eaten, and also to indicate their usual portion size by selecting from five categories (ranging from 1 to 5+ measures per day). The SCG-FFQ also contained questions on the type and amount of vitamins, minerals and food supplements taken, and dietary change and special diets/dietary restrictions, and included a section for other foods eaten which the respondent had not already included elsewhere.

All SCG-FFQs were reviewed for completeness. Data from the reviewed SCG-FFQs were processed using the scanning software package TELEForm (version 5.2, Cardiff Software, Inc., San Marcos, CA, USA) and software based on the Oracle Relational Database Management System (Version 7), which has been developed and routinely used at the University of Aberdeen. Estimated intake of specific nutrients and micro-nutrients was computed using McCance and Widdowson's The Composition of Foods (5th ed.) (Holland *et al.* 1991b) and related supplements (Holland *et al.* 1988, 1989, 1991a, 1992a,b, Holland *et al.* 1993; Chan *et al.* 1994, 1995, 1996).

Food values for the isoflavones daidzein and genistein were obtained from the newly constructed (Ritchie, 2004; Ritchie *et al.* 2005) and validated (Ritchie *et al.* 2004) Isoflavone Food Database that contains isoflavone values for nearly 7000 foods. It incorporates foods analysed by Liggins *et al.* (2000a,b) and isoflavone estimates for soy flour and protein added as bulking agents during food processing. Isoflavone contents were assigned to each of the 155 food items using the new database. Two specific food items for soy foods were included that named the following foods as soy food examples: (1) soya beans, textured vegetable protein (TVP), tofu and soya meat substitute; and (2) nut roast, nut burgers and vegetable burgers. Soya milk and other soy-based foods reported in the 'Other Foods' section were also included in the assessment of isoflavone intake.

Blood sample collection and analysis

Non-fasting blood samples were collected at the subjects' GP practice or by a research nurse. A 20 ml aliquot of peripheral blood was collected in a vacuum syringe using the Vacutainer® system and sent to the BioClin Laboratories, Cardiff for storage and analysis.

A subgroup containing the first 454 blood samples received at BioClin Laboratories were analysed for serum phyto-oestrogens. Isotope dilution gas chromatography-mass spectrometry (GC-MS) was used to analyse the isoflavones genistein and daidzein, the daidzein metabolite equol and the lignan enterolactone in serum (Morton *et al.* 1994). The detection limit for each of the four assays was 0.1 mg/l (daidzein, 0.394 nmol/l; genistein, 0.370 nmol/l; and equol, 0.417 nmol/l); data below these limits were regarded as zero (Morton *et al.* 2002).

Statistical analysis

All statistical analyses were conducted using SPSS (version 11, SPSS Inc., Chicago, IL, USA) and STATA (version 7, STATA Inc., College Station, TX, USA). Distribution analysis was performed on intakes of isoflavones, total energy and soy food groups, phyto-oestrogen serum concentrations (genistein, daidzein, equol and enterolactone) and general anthropometric characteristics (including age, height, weight, BMI, BMR (Schofield, 1985) and the energy intake:BMR ratio (EI:BMR) (Goldberg *et al.* 1991)). As the distributions of phyto-oestrogen intake and serum concentrations in the study population were markedly skewed, medians were quoted and non-parametric tests were used to estimate differences in cases and controls. A comparison of the population and BPH controls showed that there were no significant differences in either phyto-oestrogen intake and serum concentration or general subject characteristics, with the exception of age, between these two control groups; therefore the two control groups were combined. OR and 95% CI were calculated using logistic regression for four categories of intake/serum concentration using quartiles based on the distribution of controls, with the exception of soy food consumption which used two categories (consumption of soy foods *v.* no consumption of soy foods). Adjusted OR for isoflavone intake and soy food consumption were controlled for age group, total energy intake category, family history of PCa and/or breast cancer (BrCa), Carstairs Deprivation Index (a deprivation index by Carstairs & Morris, 1991, based on the 1991 census data), smoking status and the EI:BMR ratio, by including these potential confounders as co-variables in the multivariate logistic regression models. Energy-adjusted intakes for isoflavones and soy foods were also calculated using the residual method (Willett & Stampfer, 1998); these produced findings similar to those for the multivariate logistic regression model and therefore were not presented. Adjusted OR for serum phyto-oestrogens were controlled for age group, family history of PCa and/or BrCa, Carstairs Deprivation Index and smoking. Score tests for a linear trend were also conducted to examine any potential dose-response effects.

Results

A total of 604 eligible cases and 911 eligible controls were approached. Of these, 433 cases and 483 controls (including 305 population controls and 178 BPH controls) completed an SCG-FFQ and provided a blood sample. Blood samples from the first 454 subjects to provide a blood sample (249 cases and 205 controls) were analysed for serum phyto-oestrogens. A total of 916 subjects were therefore included in this study, all of which were included the nutrient/food consumption analysis, whereas 454 subjects were included in the subset analysis of serum phyto-oestrogens.

Subject characteristics

As shown in Table 1, there was very little variation in subject characteristics between cases and controls, with the exception of mean age where cases were significantly older than controls. This difference is due to the inclusion of BPH controls

that were not age frequency matched to cases, unlike population controls. The EI:BMR ratio was also significantly higher in the cases, suggesting either that cases consumed more food than necessary (thereby suggesting that overconsumption is associated with increased risk of PCa) or that the controls were under-reporting. As BMI did not differ significantly between cases and controls, the latter seems more likely; this was confirmed by the proportion of low energy responders (subjects who under-reported food consumption) being significantly higher within the control group (Table 1). There was no significant variation in either smoking status or deprivation index between cases and controls; however, significantly more cases reported a family history of PCa and/or BrCa than controls.

Phyto-oestrogen intake and serum concentrations

The distributions of total energy and phyto-oestrogen intake, and phyto-oestrogen serum concentrations are shown in Table 2. For each of the four serum phyto-oestrogens measured, the minimum value was below the level of detection (0.1 mg/l). Data for both equol and daidzein serum concentrations were missing for five subjects, whereas genistein serum concentration data were missing for seven subjects.

Mean total energy intake was significantly lower in controls than cases; phyto-oestrogen intake was also observed to be slightly lower in controls, although this difference was not significant. There were no significant differences in phyto-oestrogen serum concentrations between cases and controls, except for enterolactone, the serum concentrations for which were found to be significantly greater within the control group. Equol was detected in 121 cases (49%) (median 0.98 nmol/l, interquartile range (IQR) 0.51–1.84) and 98 controls (49%) (median 0.67 nmol/l, IQR 0.34–1.51). Overall, there was great interindividual variation in serum isoflavones, with an 8500- and 270-fold variation between minimum and maximum values in cases and controls, respectively. Serum enterolactone concentrations also varied considerably, with a 1900- and 300-fold variation in cases and controls, respectively. The variation in estimated dietary intake of isoflavones (daidzein and genistein) was also large, from 0.01 to 21.26 mg/d and from 0.01 to 42.99 mg/d for cases and controls, respectively. Consumption of soy foods was relatively low, with only 7.2% of subjects reporting eating soy foods.

Association with PCa risk

Crude and adjusted OR for phyto-oestrogen intake and serum concentrations are shown in Table 3. Phyto-oestrogen intake was observed to have no association with PCa risk, with risk estimates remaining close to null for all levels of intake. However, the consumption of soy foods was shown to have a significant protective association against PCa, which became slightly stronger when confounding factors were adjusted for (adjusted OR 0.52, 95% CI 0.30, 0.91). No significant associations were observed for phyto-oestrogen serum concentrations, with the exception of enterolactone for which significant protective associations were observed for all categories of serum concentrations compared with the lowest category, both with and without adjusting for potential confounding factors (adjusted OR for highest

Table 1. Subject characteristics by subject status

	Cases* (n 433)	Controls* (n 483)	Test for difference between cases and controls† (P value)
Age (years)	67.2 (5.5)	66.0 (5.4)	0.001
Height (m)	1.74 (0.08)	1.74 (0.07)	0.91
Weight (kg)	79.8 (13.4)	79.1 (12.1)	0.40
BMI	26.3 (4.0)	26.1 (3.6)	0.37
EI:BMR	1.59 (0.50)	1.51 (0.50)	0.03
Family history of cancer			
No family history of PCa or BrCa	298 (69)	387 (80)	0.001
Family history of PCa	60 (14)	38 (8)	
Family history of BrCa	58 (13)	48 (10)	
Family history of PCa and BrCa	17 (4)	10 (2)	
Smoking			
Non-smoker	175 (41)	229 (48)	0.11
Ex-smoker	174 (41)	168 (35)	
Smoker	76 (18)	79 (17)	
LER			
Yes	62 (15)	95 (20)	0.03
No	359 (85)	371 (80)	
Carstairs Deprivation Index			
1	69 (16)	66 (14)	0.87
2	64 (15)	73 (15)	
3	97 (23)	103 (22)	
4	75 (18)	93 (20)	
5	54 (13)	69 (15)	
6	31 (7)	33 (7)	
7	37 (9)	35 (7)	

EI:BMR, energy intake:BMR ratio; PCa, prostate cancer; BrCa, breast cancer; LER, low energy responder.

* Values for age, height, weight, BMI and EI:BMT ratio are means (SD), values for family history of cancer, smoking, LER and Carstairs Deprivation Index are frequencies (%).

† Using *t* test for age, height, weight, BMI and EI:BMR ratio, and χ^2 test for family history of cancer, smoking, LER and Carstairs Deprivation Index Percentages may not add up to 100% due to rounding.

serum category = 0.40, 95% CI 0.22, 0.71) and for which a significant dose–response effect was found ($P=0.002$).

Discussion

In this case–control study, a significant protective effect of serum enterolactone against PCa was observed. The findings for isoflavones were less clear. No association with PCa risk was observed for either serum isoflavones or isoflavone intake; however, consumption of soy foods was observed to be associated with a significant decrease in PCa risk.

The point estimate of a 60% reduction in PCa risk associated with high concentrations of serum enterolactone observed in this study is not in agreement with the three previous nested case–control studies that reported no significant associations between serum enterolactone and PCa risk in Scandinavian men (Stattin *et al.* 2002, 2004; Kilkkinen *et al.* 2003). One possible reason for the lack of significant associations observed in these previous studies is that the relatively low enterolactone serum levels reported in all three studies (median: 8.4 and 8.5 nmol/l for cases and controls, respectively (Stattin *et al.* 2002), compared with 11.78 and 16.16 nmol/l for cases and controls in this study) were too low for a protective effect to be observed. Also, the long storage times of the serum samples reported by Stattin *et al.* (2002) (a large proportion of samples were stored for >20 years) could have allowed for the degradation of serum enterolactone, thereby leading to a possible random misclassification of enterolactone concentrations which in turn may have caused an attenuation of the risk estimates towards the

null. Furthermore, Kilkkinen *et al.* (2003) examined serum enterolactone in a population comprised only of smokers. Not only does the inclusion of only smokers restrict the generalisability of the findings, but smoking is associated with both lower levels of serum enterolactone (Kilkkinen *et al.* 2001) and an increased risk of fatal PCa (Hsing *et al.* 1990, 1991). It is therefore possible that the serum enterolactone concentrations reported in this study were high and varied enough for the true protective association to be detected. Although no significant association between the intake of enterolactone precursors and PCa risk has been reported (Strom *et al.* 1999), our findings are supported by a cohort study of Adventist men (Mills *et al.* 1989) and three case–control studies (Key *et al.* 1997; Kolonel *et al.* 2000; Hodge *et al.* 2004) undertaken in Western populations including the UK (Key *et al.* 1997), which identified consumption of foods rich in enterolactone precursors, such as beans, peas and lentils, as being significantly associated with a protective effect against PCa.

Consumption of soy foods was associated with a 50% reduction in PCa risk in this study. This finding is similar to those of two prospective studies on men of Japanese ancestry living in Hawaii (Severson *et al.* 1989) and Californian Seventh-day Adventist men (Jacobsen *et al.* 1998) who reported that men who consumed tofu more than five times a week (Severson *et al.* 1989) or soy milk more than once a day (Jacobsen *et al.* 1998) had a 65% (Severson *et al.* 1989) and 70% (Jacobsen *et al.* 1998) reduction in PCa risk, respectively. Several case–control studies, conducted within populations with high soy consumption, have also

Table 2. Nutrient intake, soy consumption and phyto-oestrogen serum concentrations by subject status

Nutrient/soy food consumption	Cases (n 437)		Controls (n 483)		Test for difference between cases and controls* (P value)
	Median (IQR)	Range	Median (IQR)	Range	
Total energy (kJ/d)	10 165 (8369–12 370)	3431–27 866	9684 (7874–11 757)	1549–24 681	0.02
Isoflavones (mg/d)	1.14 (0.64–2.44)	0.01–21.26	1.05 (0.58–1.98)	0.01–42.99	0.10
Soy foods (servings per week)	0.00 (0.00–0.00)	0.0–4.0	0.00 (0.00–0.00)	0.0–15.0	0.04
Subset analysis: phyto-oestrogen serum concentrations			Controls (n 205)		
	Median† (IQR)	Range†	Median† (IQR)	Range†	
Equol (nmol/l)	0.00 (0.00–0.96)	0.00–89.24	0.00 (0.00–0.67)	0.00–59.32	0.409
Daidzein (nmol/l)	17.57 (9.38–34.46)	0.00–1030.52	18.00 (8.26–29.11)	0.00–222.53	0.506
Daidzein and equol combined (nmol/l)	18.43 (9.83–34.73)	0.00–1030.52	18.66 (8.48–33.69)	0.00–224.21	0.508
Genistein (nmol/l)	34.60 (16.34–77.52)	0.00–1942.17	33.46 (14.23–64.53)	0.00–316.02	0.223
Enterolactone (nmol/l)	11.78 (4.03–23.52)	0.00–330.29	16.16 (8.41–28.90)	0.00–537.60	0.001
Total isoflavones‡ (nmol/l)	51.41 (28.23–112.15)	0.32–2742.97	50.52 (25.57–98.86)	1.62–435.01	0.294

IQR, interquartile range

*Using Mann–Whitney U test.

†0.00=below the level of detection (0.1 mg/l).

‡Total Isoflavones = daidzein + genistein + equol.

confirmed this association (Kolonel *et al.* 2000; Lee *et al.* 2003; Chen *et al.* 2005). In contrast, a large Canadian study (Villeneuve *et al.* 1999) did not detect any association between soy food consumption and PCa risk. In addition, a recent meta-analysis (Yan & Spitznagel, 2005) conducted on eight studies that examined the association between soy consumption and PCa reported an overall risk estimate of 0.70 (95% CI 0.59, 0.83, $P < 0.001$). The relatively low consumption of soy foods meant that further analyses on soy food consumption to investigate linear trend was not possible.

However, this study observed no significant associations between either isoflavone intake or serum concentrations and PCa risk. No association was observed for isoflavone intake (OR = 1.18, 95% CI 0.41, 2.25); this is different from the findings of two case-control studies on Caucasian US men (Strom *et al.* 1999) and Chinese men (Lee *et al.* 2003) which reported a protective trend against PCa for both genistein and daidzein intake. However, of these, only the association for genistein intake within the Chinese men was found to be significant (Lee *et al.* 2003). Daily isoflavone intake within the Chinese study (Lee *et al.* 2003) was 76 mg; this is seventy times greater than the daily intake observed in this study (1.1 mg/d) and also by Strom *et al.* (1999) (1.2 mg/d). It is very possible that the intake of isoflavones observed in this study was not high enough for a protective effect to be detected. No significant associations between serum isoflavones and PCa risk were found in this study. These findings are similar to those of Akaza *et al.* (2002) who observed no significant difference in either daidzein or genistein serum concentrations between cases and controls within a Japanese population. The only other study to examine the association between serum isoflavones and PCa (Ozasa *et al.* 2004) reported a non-significant dose-dependent protective association for both daidzein and genistein serum concentrations, and a significant protective association for both equol (OR 0.39, 95% CI 0.15, 0.98) and combined equol/daidzein (OR 0.35, 95% CI 0.13, 0.89).

As with all case-control studies, this study was subjected to several methodological limitations. The methods used in the selection and recruitment of this study allowed for potential bias and confounding to be limited. The overall response rate was observed to be 67%. The response rate differed between cases and controls (80 and 63%, respectively); it is therefore possible that bias may have been introduced into the study. However, analysis of response rates showed that the observed trend towards younger subjects and those with lower levels of deprivation being more likely to respond were similar in both cases and controls. In order to protect against disease/subject misclassification, all cases were histologically confirmed as having PCa. In addition, the use of BPH controls ensured that a large proportion of controls were confirmed as unlikely to have asymptomatic PCa. Also, the combining of the two control groups (which had similar distributions of phyto-oestrogen serum and intake and confounding factors, and were observed to have similar risk estimates when compared with cases separately) increased the statistical power of the study. The use of BPH controls could introduce selection bias if BPH is associated with PCa risk; however, to date, no evidence for this association has been reported (Guess, 2001). To minimise recall bias, only

Table 3. Logistic regression analysis – crude and adjusted OR

	Frequency		Crude OR		Adjusted OR*	
	Case (n)	Control (n)	OR	(95% CI)	OR	(95% CI)
Total energy intake (kJ/d)						
0–7874	87	121	1.00	–	1.00	–
7874–9684	105	121	1.21	(0.82, 1.77)	1.02	(0.59, 1.76)
9684–11 757	106	121	1.22	(0.83, 1.78)	1.03	(0.53, 2.01)
> 11 757	135	120	1.56	(1.08, 2.27)	0.96	(0.41, 2.25)
Score test for linear trend: $P=1.00$ †						
Isoflavone intake ($\mu\text{g/d}$)						
0–581.1	95	121	1.00	–	1.00	–
581.1–1050.9	100	121	1.05	(0.72, 1.54)	1.12	(0.76, 1.67)
1050.9–1982.8	112	121	1.18	(0.81, 1.71)	1.11	(0.75, 1.65)
> 1982.8	126	120	1.34	(0.93, 1.93)	1.18	(0.79, 1.75)
Score test for linear trend: $P=0.87$ †						
Soy food consumption						
No	410	440	1.00	–	1.00	–
Yes	23	43	0.57	(0.34, 0.97)	0.52	(0.30, 0.91)
Score test for linear trend: $P=0.34$ †						
Serum equol (nmol/l)						
0	126	102	1.00	–	1.00	–
≥ 0.10	121	98	1.00	(0.69, 1.45)	1.07	(0.71, 1.61)
Score test for linear trend: $P=0.75$ †						
Serum daidzein (nmol/l)						
0.00–8.26	51	47	1.00	–	1.00	–
8.26–18.00	74	53	1.29	(0.76, 2.19)	1.20	(0.67, 2.14)
18.00–29.11	44	51	0.80	(0.45, 1.40)	0.74	(0.40, 1.37)
> 29.11	78	49	1.47	(0.86, 2.51)	1.34	(0.76, 2.38)
Score test for linear trend: $P=0.21$ †						
Serum daidzein and equol combined (nmol/l)						
0.00–8.48	47	50	1.00	–	1.00	–
8.48–18.66	81	50	1.72	(1.01, 2.95)	1.79	(1.00, 3.20)
18.66–33.69	54	50	1.15	(0.66, 2.00)	1.01	(0.56, 1.85)
> 33.69	65	50	1.38	(0.80, 2.39)	1.34	(0.75, 2.40)
Score test for linear trend: $P=0.14$ †						
Serum genistein (nmol/l)						
0.00–14.23	49	49	1.00	–	1.00	–
14.23–33.46	68	50	1.36	(0.79, 2.34)	1.38	(0.76, 2.50)
33.46–64.53	48	50	0.96	(0.55, 1.68)	0.90	(0.49, 1.67)
> 64.53	73	49	1.49	(0.89, 2.56)	1.36	(0.76, 2.43)
Score test for linear trend: $P=0.37$ †						
Serum enterolactone (nmol/l)						
0.00–8.41	108	50	1.00	–	1.00	–
8.41–16.16	40	50	0.37	(0.21, 0.64)	0.43	(0.24, 0.76)
16.16–28.90	53	50	0.49	(0.29, 0.83)	0.47	(0.27, 0.81)
> 28.90	46	50	0.43	(0.25, 0.73)	0.40	(0.22, 0.71)
Score test for linear trend: $P=0.002$ †						
Total serum isoflavones‡ (nmol/l)						
0.00–25.57	54	49	1.00	–	1.00	–
25.57–50.52	63	50	1.14	(0.67, 1.96)	1.09	(0.60, 1.96)
50.52–98.86	49	50	0.89	(0.51, 1.55)	0.85	(0.46, 1.55)
> 98.86	72	49	1.33	(0.78, 2.27)	1.24	(0.69, 2.20)
Score test for linear trend: $P=0.64$ †						

* Isoflavone intake and soy food consumption adjusted for age, total energy intake, family history of PCa and BrCa, Carstairs Deprivation Index, smoking and energy intake:BMR ratio. Serum phyto-oestrogens adjusted for age, family history of prostate cancer and breast cancer, Carstairs Deprivation Index and smoking.

† Adjusted OR.

‡ Total isoflavones = daidzein, genistein and equol.

newly diagnosed cases were used. However, due to recent media attention on the benefits of soy foods, it is still possible that not only did some cases recall their dietary habits differently, but also that they could have changed their diets post-diagnosis to increase consumption of these foods. This could lead to differential misclassification of isoflavone and soy food intake, and may be the reason behind why a slight positive association with PCa risk was observed in the highest categories of both isoflavone intake and serum concentrations.

The self-completion of the SCG-FFQ allowed for observer bias to be minimised. The use of SCG-FFQ, which has been validated against serum isoflavones within the population under study (Heald *et al.* 2005), and the new comprehensive Isoflavone Food Database – constructed and validated within the target population under study (Ritchie, 2004; Ritchie *et al.* 2004) – also allowed for accurate estimations of isoflavone intake, thereby minimising any further potential misclassification of intake.

To minimise the effects of potential confounders, known risk factors were included in the multivariate regression model; however it is likely that residual confounding due to unmeasured or unknown factors may have remained. It is possible that dietary co-variables of phyto-oestrogens may be responsible for the observed associations with PCa risk. As the plant precursors of enterolactone are predominantly found in whole-grain products, legumes, seeds, fruits and vegetables, it may be that serum enterolactone is a proxy for a general healthy diet. This could also be the case for soy foods, as this food group is also associated with a healthy diet rich in fruit and vegetables and low in animal products – a dietary factor observed to increase PCa risk (Kolonel, 2001). In addition to dietary intake, serum phyto-oestrogen levels are also influenced by gut microflora metabolism and absorption from the gut (Rowland *et al.* 1999, 2000; Kilkkinen *et al.* 2001). Absorption and metabolism of phyto-oestrogens has been reported to be influenced by high fat intake (Stumpf *et al.* 2000; Rowland *et al.* 2000; Kilkkinen *et al.* 2001) and also by antibiotic use (Kilkkinen *et al.* 2002), most probably due to their effect on the gut microflora.

The observed associations between serum phyto-oestrogens and PCa risk may also have been influenced by the use of just one serum sample for each subject. A moderately high level of reliability over 2 years has been reported for serum enterolactone measurements (reliability coefficient = 0.55), but not for isoflavone serum measurements (reliability coefficients ≤ 0.30) (Zeleniuch-Jacquotte *et al.* 1998). This level of reliability of serum enterolactone measurements suggests a reasonable degree of long-term stability for intra-individual serum enterolactone levels, thereby suggesting that one serum measurement may be sufficient to estimate long-term average levels for epidemiological studies, although estimated relative risks may be attenuated. The lack of long-term reliability for isoflavone serum measurements may be another reason for the absence of an observed association between serum isoflavone concentrations and PCa risk in this study, as the one serum measurement used in this study may not be an accurate indicator of long-term serum levels of isoflavones. However, the lack of an observed association between isoflavone intake and PCa risk may confirm a true lack of effect on PCa risk for serum isoflavones.

In conclusion, our findings on a population of Scottish men support the hypotheses that serum enterolactone and soy food consumption protect against PCa risk. Interestingly enough, an association between PCa risk and isoflavone intake or serum concentrations was not found. In the light of these findings, future retrospective studies investigating the association between PCa risk and phyto-oestrogen intake and serum should include subjects with a greater variation of phyto-oestrogen and/or soy food intake. Such studies should also assess dietary intake of lignan precursors and its potential relationship with PCa risk.

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